LATE-PCR for LoC molecular diagnostics devices and its application to the sensitive detection of SARS-CoV-2

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The current pandemic caused by the Severe Acute Respiratory Syndrome-coronavirus-2 (SARS-CoV-2) has stressed the need for testing on a massive scale. Towards this goal, numerous efforts have been made to fabricate reliable and cost-efficient screening devices that can be deployed at the Point-of-Need. All of these approaches suffer from low sensitivity, repeatability and high operation cost due to the use of highly specialized reagents and primers (LAMP, and difficulty in multiplexing. such as immunoassays (Rapid-Tests, ELISA etc.) but also molecular-based methods primarily based on isothermal amplification as a means to substitute for the "golden standard" diagnostic procedure, qPCR, since the latter are much more easily incorporated into portable devices. Nevertheless, all of these approaches suffer from low sensitivity, repeatability and high operation cost due to the use of highly specialized reagents and primers (LAMP, RPA), while multiplexed analysis in a single chamber is impossible thus increasing the footprint of the portable device.

Herein we report the development of a detection protocol for SARS-CoV-2 through the design of appropriate primers that target evolutionary conserved regions of the virus based on an improved version of asymmetric RT-PCR, the Linear-After-The-Exponential (LATE)-PCR that uses primers deliberately designed for use at unequal concentrations. LATE-PCR exhibits similar efficiency to symmetric PCR while promoting accumulation of single-stranded products that can subsequently hybridize to a single strand DNA probe-spotted microarray. The performance of the developed LATE-PCR protocol was compared to that of symmetric RT-PCR and validated with the use of artificial viral RNA and nasopharyngeal swabs samples from real patients. Furthermore, and in order to illustrate its potential for integration into a biosensor platform, the amplicons were allowed to hybridize with probes covalently immobilized onto commercially-available functionalized glass, without the need of heat denaturation.

RNA extracted from positive samples

Artificial SARS-CoV-2 RNA

Primer design

Pasteur recommended Primer/Probe Set for IP2 gene

Forward Primer (limiting)

Modified Forward Primers

+1 +2 +3 +4

Reverse Primer (excess) (1000μM)

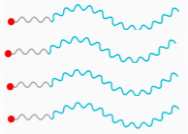
Cycling Program

- 50min at 55°C (RT)
- 3 minutes at 95°C (denaturation)
- 45 cycles at 95°C,12 sec
- 58°C for 30 sec.

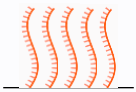
Analysis

Reverse Primer Tagged with Cy5

Visualization



30 min RT incubation

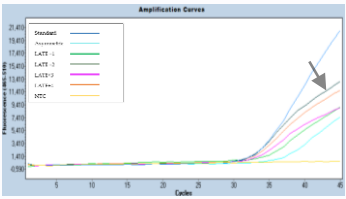


2D carboxy-terminated glass slides (PolyAn), Activated with EDC-NHS Spotted with complementary amino-modified probes

Real Time PCR

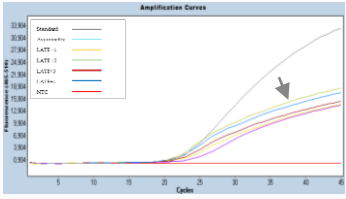
Positive human sample

Protocol	Cp	SD
Standard	23	0.17
Asymmetric	24.23	0.09
LATE +1 base added	22.88	0.2
LATE +2 bases added	20.96	0.15
LATE +3 bases added	22.09	0.16
LATE +4 bases added	21.55	0.11



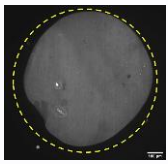
Artificial RNA

Protocol	Cp	SD
Standard	33.80	0.13
Asymmetric	37.02	0.02
LATE +1 base added	34.58	0.26
LATE +2 bases added	32.46	0.12
LATE +3 bases added	33.16	0.07
LATE +4 bases added	32.79	0.09



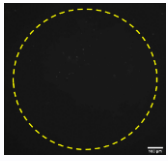
Array Detection

LATE-PCR Protocol



ssDNA hybridizes to immobilized probes

Standard Protocol



dsDNA product does not hybridize

- ✓ **LATE-PCR effectively eliminates the need for multiple rounds of amplification or laborious steps for single-stranded DNA generation**
- ✓ **The need for mechanical or enzymatic separation and purification of single-stranded DNA becomes obsolete**
- ✓ **Ideally suited as an amplification technique for Point-of-Need LoC Devices, especially when coupled with DNA microarray detection**
- ✓ **Multiplex detection is feasible without the need for multiple reservoirs and chambers on a microfluidic cartridge**

NextGen Microfluidics

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